Ontogeny of the Epidermal Permeability Barrier

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A competent permeability barrier must be present by the end of gestation to allow for life in a terrestrial environment. Indeed, early preterm infants display serious complications of skin immaturity. Yet, regardless of their degree of prematurity, all infants quickly develop a competent barrier. To learn more about the mechanisms and regulation of barrier ontogeny, we have utilized late-gestation fetal rodents. In 19–21 d fetal rats, we showed that barrier competence is accompanied by both enhanced epidermal development and formation of extracellular lamellar membranes in the stratum corneum. The identical sequence and time-course occurs when fetal rat skin is cultured in a serum-free medium. Glucocorticoids, thyroid hormone (T_3), and estrogen accelerate, while androgens delay barrier formation both *in utero* and in the *in vitro* system, explaining the poorer outcome of premature males *versus* females. But neither T_3 nor glucocorticoids are absolutely required for barrier development. Lifting fetal skin cultures to an air–medium interface also accelerates barrier formation, explaining the rapid emergence of barrier competence in very premature infants. PPARα and FXR activators, which, like T_3, heterodimerize with the nuclear receptor, RXR, also accelerate barrier development *in vitro*. Finally, not only the nuclear receptor family, but also Ca^{++} could regulate key events late in barrier development. ***Key words: epidermal development/fetal skin/FXR/lipid metabolism/permeability barrier/PPARα/regulation/RXR. Journal of Investigative Dermatology Symposium Proceedings 3:75–79, 1998***

A competent barrier to systemic water loss is an essential requirement for terrestrial life; hence, a mature barrier must be present by the end of normal gestation. Full-term human newborns, in fact, possess a competent barrier at birth, with rates of transepidermal water loss (TEWL) at least as low as in adults. In contrast, preterm infants (<33 wk gestational age) exhibit both increased TEWL and enhanced percutaneous absorption of xenobiotics, in proportion to their degree of prematurity (Nachman and Esterly, 1971; Hammarlund and Sedin, 1979; Rutter and Hull, 1979). Moreover, skin immaturity can be a major source of morbidity in the very premature infant (<28 wk), causing hypothermia and increased caloric requirements due to evaporative heat loss, fluid and electrolyte imbalance from excessive TEWL, and transcutaneously acquired infections (Belgaumkar and Scott, 1975; Lorenz *et al*, 1982; Maurer, 1984). Two additional clinical features of barrier formation are recognized (Rowan *et al*, 1995; Nopper *et al*, 1996: (i) barrier formation in intrauterine growth-retarded infants is appropriate to their gestational ages (Hammarlund and Sedin, 1979); and (ii) regardless of the initial degree of prematurity, all surviving infants develop a competent barrier by ≈2 wk postgestation (Evans and Rutter, 1986; Barker *et al*, 1987).

Whereas cornification in humans begins by 14–15 wk in a folliculocentric pattern, proceeding in a cephalo-caudad direction, interfollicular cornification does not begin until ≈24 wk (Cartlidge and Rutter, 1992). The third trimester is characterized by a progressive increase in the thickness of all skin layers, including the stratum corneum (SC). Yet, despite its clinical importance, until recently little was known about the biochemical processes underlying permeability barrier ontogenesis, beyond the descriptive outline provided above. In postnatal skin, the permeability barrier resides within the SC interstices, comprising multiple, hydrophobic membranes organized into lamellar unit structures (Elias, 1983; Elias and Menon, 1991). The lipids that comprise these membranes are predominantly ceramides, cholesterol, and free fatty acids (Schürer *et al* 1991), which derive largely from local epidermal synthesis (Feingold, 1991). These lipids are delivered as a polar lipid mixture to the extracellular domains of the SC through exocytosis of the contents of the epidermal lamellar body, which occurs at the stratum granulosum–SC interface (Elias and Menon, 1991). After secretion, lamellar body-derived glucosidases and lipases convert glucosylceramides to ceramides and phospholipids to free fatty acids, respectively. The cholesterol in the SC derives both from cholesterol, delivered unchanged with lamellar body contents, and from the hydrolysis of cholesterol sulfate to cholesterol within SC extracellular domains.

LESSONS FROM FETAL RODENT MODELS

In utero and *in vitro* models Our group has utilized the fetal rat as a convenient model for the study of mammalian barrier ontogenesis. In our initial studies we demonstrated that the barrier also forms during late gestation in the fetal rat; i.e., on day 20, such that by day 21 of gestation all pups demonstrate a competent barrier to excess water loss (term = 22 d) (Aszterbaum *et al*, 1992). Development of a competent barrier is accompanied by the formation of a multilayered SC on light microscopy, and by the deposition of neutral lipid in a membrane pattern in the SC, as viewed by fluorescence microscopy, using the hydrophobic probe, nile red (Aszterbaum *et al*, 1992). Ultrastructural studies, using ruthenium tetroxide (RuO_4) postfixation to visualize SC membranes, reveal that lamellar bodies are generated and secreted into the SC interstices prior to barrier formation (i.e., on day 19), but these extracellular lipids do not undergo transformation into lamellar unit structures until days 20–21, coincident with the formation of the barrier

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Abbreviation: SC, stratum corneum; TEWL, transepidermal water loss.

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(Aszterbaum *et al*, 1992). Lipid analytical studies also demonstrated that barrier formation is accompanied by the progressive enrichment of the SC in cholesterol and ceramides, lipids that along with long-chain, free fatty acids, are the predominant constituents of mature SC membranes (Aszterbaum *et al*, 1992).

Fetal rat skin obtained on day 17 and maintained submerged in a defined, serum-free medium, undergoes morphologic and functional maturation on a timetable that is virtually identical to *in utero* barrier ontogenesis (= explant model) (Hanley *et al*, 1996a). After 4 d *in vitro*, corresponding to day 21 of gestation, a multilayered SC is present that displays mature lamellar unit structures on electron microscopy with ruthenium tetroxide (RuO_4) postfixation, and a competent barrier to water loss (Hanley *et al*, 1996a). When fetal rat skin is cultured on rafts at the air–medium interface, barrier formation accelerates, with a competent barrier forming after 2 d *in vitro* (= day 19 gestation), instead of the usual 4 d (17 + 4) in the immersed controls (Hanley *et al*, 1997a). Because acceleration of ontogenesis in the lifted fetal rat organ cultures can be prevented by occlusion of the epidermal surface with water vapor-impermeable membranes, but not with water vapor-permeable membranes, water flux may signal accelerated barrier formation (Hanley *et al*, 1997a). This mechanism also could explain the accelerated barrier development that occurs following premature birth in humans. Thus, the explant model mirrors both *in utero* development when submerged, and accelerated maturation following premature birth.

The calcium gradient The epidermal calcium (Ca^{++}) gradient is postulated to regulate barrier homeostasis in the mature animal (Lee *et al*, 1992). When the barrier is perturbed by either solvent treatment or tape stripping, the high Ca^{++} milieu of the outer nucleated layers of the epidermis is lost (Menon *et al*, 1992). This decrease in Ca^{++} concentration induces a series of responses in the epidermis, including the rapid secretion of a preformed pool of lamellar bodies from the outermost granular cells (Menon *et al*, 1992), and an increase in lipid synthesis (Lee *et al*, 1992), both of which are essential for barrier recovery. Furthermore, using sonophoresis, we showed that changes in Ca^{++} itself, rather than barrier disruption, modulate lamellar body secretion: lamellar body secretion occurs when a low Ca^{++} (but not a high Ca^{++}) solution is sonophoresed into the epidermis, a process that does not disrupt the barrier (Menon *et al*, 1994). As the barrier reforms, the Ca^{++} gradient is reestablished and the lamellar body pool is restored within the outermost stratum granulosum cell.

To determine whether the Ca^{++} gradient forms during fetal barrier maturation in rodents, we utilized two independent methods, ion capture cytochemistry and proton emission X-ray spectroscopy (PIXE). Both systems demonstrate that the Ca^{++} gradient forms at the same time as the functional barrier is established (Elias *et al*, 1998). Lipid synthesis and lamellar body secretion peak prior to barrier formation; i.e., prior to generation of the Ca^{++} gradient, whereas conversely, lipid synthesis and lamellar body secretion decline as the barrier forms and the Ca^{++} gradient appears. Moreover, key late differentiation markers, such as cornified envelope and epidermal transglutaminase 1 activity, increase in parallel with the appearance of the Ca^{++} gradient (Hanley *et al*, in preparation). Although these data are correlative, they nevertheless suggest that certain key steps in barrier formation and terminal differentiation are linked to modulations in the Ca^{++} gradient (**Table I**).

The mechanism whereby the Ca^{++} gradient is established is not known. One theory holds that it arises passively, as free water evaporates from the skin surface. These fetal studies raise the possibility, instead, that the gradient formed actively, because minimal transcutaneous water movement would occur during fetal life, when the epidermis is bathed in an isotonic milieu.

Intrauterine growth retardation and the permeability barrier The intrauterine growth retarded fetal rat provides additional validation of the fetal rat model (Williams *et al*, 1993). As noted above, small for gestational age infants nevertheless display barrier function that is appropriate for their gestational age, rather than their birth weights (Hammarlund and Sedan, 1980). Similarly, when intrauterine, growth-retarded fetal rats are generated through unilateral ligation of the uterine artery, they develop a competent barrier to water loss by day 21, comparable with their full-sized, contralateral littermates, despite body weights more characteristic of day 19 gestation, when no barrier normally is present (Williams *et al*, 1993). Although the epidermis is much thinner in growth-retarded fetal rats, and the SC is reduced to two or three layers (Hoath *et al*, 1990), ultrastructural studies demonstrate that SC lipids are organized into mature lamellar unit structures throughout the SC interstices (Williams *et al*, 1993). These data provide further evidence that it is the organization of extracellular lipids into mature membrane structures in SC, and neither the mere presence of lipids in SC interstices nor the reduplication of SC layers, that determines barrier competence.

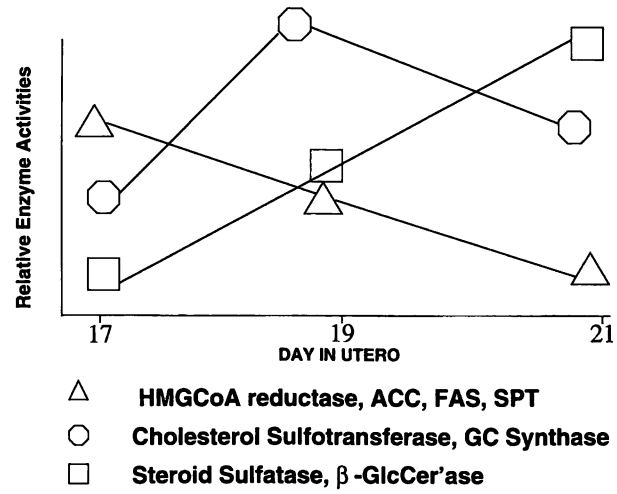

Figure 1. Modulations in lipid synthetic and processing enzymes during barrier ontogenesis in the fetal rat.

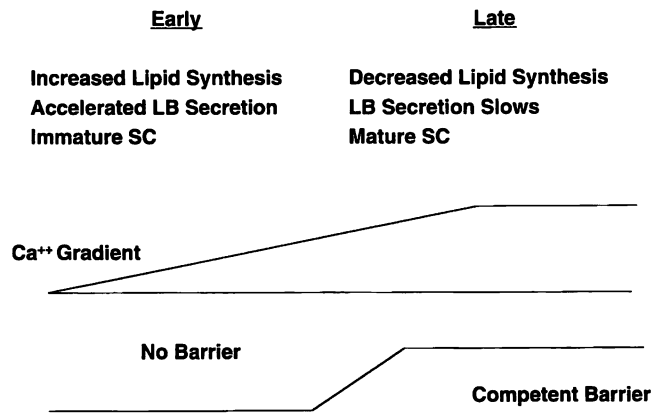

Figure 2. Potential relationship of Ca++ gradient to milestones of barrier development.

Table I. Potential regulation of milestones in fetal rat barrier development by Ca^{++} and nuclear receptors

Markers of barrier development: Regulatory mechanism	Early (≤ day 19) None	Late (≥ day 20) Competent
A. *Ca^{++} Gradient*	None	Present
Lipid synthesis	Peaks	Declines
Lamellar body secretion	Peaks	Decreases
Epidermal differentiation	Immature SC	fully formed SC
B. *Other/unknown*		
Mature extracellular lamellae	None	Present
Processing enzymes (activity)	Low	High
SC structural proteins	Low	High

Table II. RXR interacting receptors display variable effects on epidermal barrier development

Receptor	Ligands (L)/activators (A)	Barrier development
RAR	All-trans retinoic acid	None
Thyroid receptor	Thyroxine (T_3)	Accelerates
Vitamin D receptor	1, 25 $(OH)_2$ Vitamin D_3	None
LXR	Oxidized sterols (C ≤ 24)	Not determined
PPARα	Leukotriene B4; Free fatty acids; Clofibrate	Accelerates
PPARδ	Prostaglandin J2; Troglitazone	None
FXR	Farnesol, Juvenile hormone III	Accelerates

Hormonal regulation of barrier development (Table II) With models in hand, we next turned our attention to delineating the factors that regulate barrier maturation. Our initial studies were based upon prior work on the regulation of lung surfactant maturation, which displays certain intriguing similarities to the epidermis (Ballard, 1989). Lung surfactant and SC membrane lipids, although different in composition (surfactant is predominantly composed of the phospholipid, dipalmitolyphosphatidylcholine, whereas SC lipids are composed predominantly of ceramides, cholesterol, and free fatty acids), share three important features: (i) both systems mature at precisely the same time in late gestation in rodents and man (Hodson, 1992); (ii) both comprise lipids that are delivered to the extracellular domains via a lamellar body secretory system (Rooney *et al*, 1994); and (iii) both tissues are exposed to an air–medium interface.

Because of the major contribution of lung immaturity to the morbidity and mortality of prematurity, ontogenesis of the lung surfactant system has been the subject of intense study for decades. Ballard *et al* (1989) first demonstrated that glucocorticoids accelerate fetal lung surfactant maturation, and subsequently, a number of hormones (including thyroid hormone, estrogens, androgens, prolactin), and growth factors [such as epidermal growth factor (EGF), insulin-like growth factor (IGF), and transforming growth factor-a (TGF-a)], as well as cytokines (such as interferon-α), have been shown to regulate lung development (Batenburg, 1992).

Because glucocorticoids have been the most extensively studied hormones in lung maturation, and are used in the clinic to stimulate lung maturation when a premature delivery is impending, we first examined the effects of glucocorticoids on fetal rat barrier formation following intraperitoneal administration to gravid rats. Maternal glucocorticoid treatment indeed accelerates barrier formation in the fetal rat, producing a multilayered SC, with a mature pattern of lipid deposition, and a competent barrier to water loss develops by day 19 of gestation (Aszterbaum *et al*, 1993). Moreover, using the submerged, *in vitro* model of barrier development, we also observed comparable, accelerated maturation in response to exogenous glucocorticoids, such that a morphologically mature epidermis, with a competent barrier, develops after only 2 d in organ culture (normal ontogenesis = 4 d *in vitro*) (Hanley *et al*, 1996a). In contrast, addition of glucocorticoids to skin organ cultures, lifted to the air–medium interface, did not accelerate barrier formation further (Hanley *et al*, 1997a). These results suggest that factors that accelerate barrier formation *in utero* may not have clinical utility for accelerating barrier development, when administered after birth.

The *in vitro* model also has permitted a rapid evaluation of the effects of a variety of other hormones and growth factors on barrier maturation (**Table II**), agents that may be difficult to deliver across the placental barrier *in vivo*. Thus, using the *in vitro* explant model, we demonstrated that thyroid hormone also accelerates epidermal maturation, with a competent barrier forming after 2 d in culture, accompanied by the morphologic and ultrastructural features of barrier maturity (Hanley *et al*, 1996a). In contrast, a variety of other growth factors and vitamins, several of which have been shown to accelerate lung maturation, did not accelerate barrier formation *in vitro*. The agents tested and found to have no effect in this model system include: (i) several peptide growth factors and cytokines, i.e., IGF-1, EGF, KGF, TGF-a, TGF-β1, IL-1α, and TNF-α; (ii) the retinoids, all trans-retinoic acid and 9-cis retinoic acid; and (iii) 1, 25 dihydroxyvitamin D_3 (Hanley *et al*, 1996a). The absence of an effect on barrier maturation following addition of a growth factor, hormone, or vitamin to the incubation medium, however, does not exclude a potential role for that substance in barrier ontogenesis, because these tissues may have been exposed previously to sufficient levels of this factor *in utero* to permit barrier maturation to proceed *in vitro*. Moreover, some of these factors could be generated locally during organ culture in quantities sufficient to maximize their effect on barrier maturation.

Although these studies demonstrated a role for pharmacologic levels of glucocorticoids and thyroid hormone in accelerating barrier formation, they did not address the role of either of these hormones in normal barrier formation. The ability of 17 d fetal skin to mature during *in vitro* cultivation in a defined, hormone- and growth factor-free medium suggests that these hormones may not be absolutely required for normal barrier ontogenesis. To examine this question further, we have collaborated with two groups who have developed murine models of fetal glucocorticoid and thyroid hormone deficiency, respectively. The fetal model of hypothyroidism is due to a mutation in the TSH receptor, whereas the glucocorticoid-deficient model bears null corticotropin releasing hormone (CRH) alleles (CRH knock-out mice). In both of these models, mothers and their pups are both homozygous for the hormone-related alleles, thereby producing a severe, hormone-deficient fetal milieu (Hanley *et al*, 1997c, d). Moreover, in both models, SC development is delayed *in utero*, although by term, both of the hormone-deficient models displayed normal SC maturation. Thus, both glucocorticoids and thyroid hormone play a physiologic role in normal barrier maturation, and yet neither hormone is absolutely essential for barrier formation. Furthermore, treatment of hypothyroid pups with glucocorticoids neither accelerated or normalized SC maturation (Hanley *et al*, 1997c), suggesting that glucocorticoids may act via thyroid hormone (or alternatively, glucocorticoid action may require the euthyroid state).

We also examined sex differences and the role of the sex hormones, estrogen and testosterone, in fetal barrier maturation. It has long been recognized that male sex portends a poorer outcome for premature infants. Moreover, sex differences in morbidity and mortality continue to be observed, despite mitigation of delayed lung immaturity in males by surfactant replacement (Allen *et al*, 1993; La Pine *et al*, 1995). These observations suggest that cutaneous maturation may be delayed in males and contribute to their poorer outcomes. To examine the role of skin immaturity in males, we measured TEWL in male *versus* female rat pups on day 20 of gestation and observed a significantly poorer barrier in male pups (Hanley *et al*, 1996b). To evaluate the role of sex hormones in this sexual dimorphism, we exposed fetal rat skin to either estrogens or androgens *in utero* by maternal injections, or *in vitro* in submerged organ cultures. Estrogens accelerate barrier formation both *in utero* and *in vitro*, and as previously observed in the other models of accelerated barrier maturation, a competent barrier is accompanied by increased SC thickness, deposition of lipids in a membrane pattern in the SC, and formation of mature lamellar unit structures (Hanley *et al*, 1996b). In contrast, testosterone delays barrier maturation both *in utero* and *in vitro*. Whereas little difference in SC morphology is evident at a light microscopic level, ultrastructurally, SC membranes are immature in androgen-treated fetal skin; i.e., there is a delay in the reorganization of initially secreted lamellar material into lamellar unit structures (Hanley *et al*, 1996b). To determine whether the delayed barrier formation in male pups is due to an inhibitory effect of endogenous androgens on skin maturation in males, or instead to an stimulatory effect of endogenous estrogens in females, we also treated pregnant rats with the anti-androgen flutamide. Flutamide-treatment abolished the sex difference in barrier maturation (Hanley *et al*, 1996b). These studies suggest that, like glucocorticoids and thyroid hormone, pharmacologic treatment with estrogens accelerates barrier formation. Whether estrogens play a physiologic role in barrier formation is unknown; however, physiologic levels of androgens clearly are responsible for delayed barrier maturation in males. These studies suggest that the relatively greater immaturity of barrier function in males may contribute to the greater neonatal morbidity and mortality of premature male infants.

REGULATION BY ACTIVATORS AND LIGANDS OF THE NUCLEAR HORMONE SUPERFAMILY

Introduction Differential control of gene expression is essential for tissue differentiation and development. Lipophilic compounds, such as steroid hormones, thyroid hormones, vitamin D, and retinoids, are potent regulators of differentiation and development, which are mediated by cytoplasmic/nuclear receptors (Mangelsdorf *et al*, 1995). These compounds bind to their respective receptors, and interact with DNA to either induce or repress the expression of a large number of different genes. The nuclear receptors are all characterized by a central DNA binding domain, which targets the receptor to specific DNA sequences (response elements). The C-terminal portion of the receptor includes the ligand binding domain that recognizes specific hormones, vitamins, retinoids, or other lipophilic compounds. The interaction of a ligand with its specific receptor shifts the receptor to a transcriptionally active state, which allows the DNA binding domain to regulate gene expression. Currently, over 150 different members of this receptor family have been identified, making this group of nuclear receptors the largest known family of transcription factors (Mangelsdorf *et al*, 1995). Whereas the activators or ligands for some of these receptors are known, in many instances they are unknown (orphan receptors).

The nuclear receptor superfamily has been divided into four major subgroups, according to their dimerization and DNA binding properties (Mangelsdorf *et al*, 1995). Class I receptors consist of the classic steroid receptors (glucocorticoid, mineral cocorticoid, progesterone, androgen, and estrogen receptors), which are localized in the cytoplasm in association with heat shock proteins, and which translocate to the nucleus after binding their hormonal ligand. These ligands function as ligand-induced homodimers, which bind to DNA half sites organized as inverted repeats. As shown above, three ligands for this class of receptors; i.e., glucocorticoids, estrogens, and androgens, regulate barrier ontogenesis. Class II receptors consist of nuclear receptors, which heterodimerize with RXR, and usually bind to direct repeats separated by a variable number of spacer nucleotides. The ligands in this family are chemically diverse and include hormones, vitamins, and products of cellular metabolic pathways that may be produced within the cell (**Table III**). Whereas this class includes one ligand, T_3, which we have already shown regulates barrier ontogenesis, ligands for the vitamin D receptor, RAR, and RXR had no effect (**Table III**). Class III receptors bind directly to DNA repeats as homodimers, and include receptors for both known ligands, such as RXR (ligand = 9-cis retinoic acid) and orphans (e.g., HNF-4 and COOP/ARP). With the exception of RXR, receptor ligands of this class have not been studied for their ability to influence barrier development. Class IV receptors, which are all orphans, bind as monomers to a single hexameric core recognition motif flanked by additional sequences upstream of this motif.

Newly identified nuclear receptors that regulate barrier ontogenesis PPAR and FXR Epidermis is a highly active site of both cholesterol and fatty acid synthesis, which is relatively autonomous from regulation by circulating lipids. We therefore hypothesized that epidermis might possess endogenous regulatory mechanism(s) to control permeability barrier ontogenesis. Two potential candidates, PPAR and FXR, have emerged from this appraisal. Three different PPAR have been identified in mammals: alpha, delta, and gamma (Schoonjans *et al*, 1996) (**Table IV**). PPARα is expressed abundantly in liver, heart, kidney, and brown adipose tissue (Braissant *et al*, 1996; Schoonjans *et al*, 1996), and we have recently shown that PPARα mRNA is present in fetal epidermis. A wide variety of different fatty acids and drugs (e.g., clofibrate) induce peroxisomal proliferation, a marker for PPARα activation (Schoonjans *et al*, 1996). PPARα-RXR complexes stimulate the expression of a large number of different proteins, many of which are important for lipid metabolism (Schoonjans *et al*, 1996) [e.g., cytochrome P450 enzymes (CYP4 A), HMG CoA synthase, fatty acid binding protein, lipoprotein lipase, apolipoprotein A1, etc.].

The FXR receptor was cloned from liver in 1995 (Forman, 1995), and has also been demonstrated in gut, kidney, and adrenal gland tissues, which all exhibit high levels of cholesterol synthesis (**Table V**).

Table III. Pharmacologic modulation of barrier ontogenesis in the fetal rat

Stimulates	No Effect	Delays
Glucocorticoids	EGF, KGF, NGF, TGFα, IL-1α, TNFα	Androgens
Thyroxine	1, 25 $(OH)_2D_3$	
Anti-androgens (in males)	All-trans-RA, 9-cis-RA	
Farnesol, juvenile hormone	Mevalonate, squalene, 25-OH-Chol	
Selected FFA, fibrates		

Table IV. Characteristics of PPAR

PPARα	liver, heart, kidney, intestine, brown adipose tissue
PPARδ	ubiquitous
$PPAR\gamma_1$	ubiquitous (not identified yet in epidermis)
$PPAR\gamma_2$	adipose tissue

Table V. Characteristics of FXR

Closely related to ecdysone receptor
Farnesol (F) is an intermediate in cholesterol synthesis
F is an activator of FXR (actual ligand is not known)
F metabolized to juvenile hormone in insects (also activates FXR)
F interacts to form active heterodimer with RXR

This receptor, which also complexes with RXR, is activated by both the insect hormone, juvenile hormone III (JH III), and farnesol, an isoprenoid intermediate in the cholesterol biosynthetic pathway (Forman *et al*, 1995). Other intermediates in the cholesterol biosynthetic pathway, such as squalene and mevalonate, and sterol products, such as cholesterol, bile acids, and sterols, display minimal effects on FXR. Yet, because neither farnesol nor JH III bind directly to FXR, both must be considered activators, rather than ligands.

Effect of PPARα and FXR activators on fetal epidermal development and permeability barrier formation Utilizing the rat fetal skin explant model, we recently demonstrated that activators of both PPARα (e.g., oleic acid, linoleic acid, clofibrate, ETYA, and WY 14, 643) and FXR (i.e., farnesol, JH III) accelerate SC maturation and barrier formation, as evidenced by decreased TEWL, increased epidermal stratification (**Table II**), and the appearance of mature lamellae in the extracellular spaces of a multilayered SC (Hanley *et al*, 1997b). In contrast, neither PGJ2 and troglitazone (PPARγ activators), nor other sterol intermediates/metabolites, such as 25-OH-cholesterol, squalene, mevalonate, and cis-farnesol (the active compound is trans-farnesol), accelerated fetal barrier development. Additionally, PPARα and FXR activators stimulated the activities of both β-glucocerebrosidase and steroid sulfatase, two key enzymes required for normal barrier homeostasis, which usually appear during the final stages of epidermal development (see below). These studies support the concept that functional, morphologic, and enzymatic markers of barrier development are accelerated by PPARα and FXR activators. Because both fatty acid and cholesterol synthesis are very active in fetal rat epidermis prior to barrier formation, high endogenous levels of fatty acids (activators of PPARα) and farnesol (sterol intermediate and activator of FXR) are likely to be generated, becoming potentially available for autocrine/intracrine regulation. It should be noted, however, that essential fatty acids are among the most potent activators of PPARα, and these are not generated *in situ*.

EXPRESSION AND ACTIVITY OF LIPID ENZYMES DURING BARRIER ONTOGENESIS

While delineating the factors that regulate barrier maturation, we have also searched for potential targets of regulation, first focusing on key enzymes of epidermal lipid synthesis and metabolism. In the mature animal, extensive evidence suggests that the cholesterol, fatty acids, and ceramides, destined for the barrier, are generated largely by *de novo*

synthesis in the epidermis (Feingold, 1991). 3-Hydroxy-3-methyl glutaryl (HMG) Co-A reductase is the rate-limiting enzyme of cholesterol synthesis. Evidence is accumulating that cholesterol sulfate, although present in lesser quantities than the other lipid classes in SC, also may provide an important precursor pool of cholesterol for the barrier (Zettersten *et al*, 1998). The synthesis of cholesterol sulfate from cholesterol is catalyzed by the enzyme, cholesterol sulfotransferase, and cholesterol sulfate is hydrolyzed to cholesterol by steroid sulfatase in the outer epidermis. Abundant, nonessential fatty acids also are synthesized in the epidermis (Feingold *et al*, 1991). The key regulatory enzymes of fatty acid synthesis are acetyl Co carboxylase and fatty acid synthase. Fatty acids in the barrier derive in a large part from the degradation of lamellar body-derived phospholipids (Mao-Qiang *et al*, 1995), by an as yet unidentified, secretory phospholipase A_2. Ceramides in the SC are derived in large part from lamellar body-derived glucosylceramides. Serine palmitoyl transferase catalyzes the first committed step of ceramide biosynthesis; glycosylceramide synthase catalyzes the conversion of ceramides to glucosylceramides, the first committed step in glycolipid synthesis; while β-glucocerebrosidase (β-GlcCer'ase) hydrolyzes the conversion of glucosylceramides to ceramides in the outer epidermis (Menon and Elias, 1992). Our studies revealed the following pattern of enzyme activity, protein, and/or m-RNA expression during fetal rat barrier ontogenesis (**Fig. 1**): enzymes of lipid synthesis (HMG CoA reductase, acetyl Co carboxylase, FAS, and serine palmitoyl transferase) peak in epidermis prior to formation of the barrier; i.e., on or prior to day 17 in the fetal rat (Hurt *et al*, 1995), whereas glycosylceramide synthase and cholesterol sulfotransferase both peak somewhat later; i.e., around day 19, but still prior to the emergence of barrier competence (Hanley *et al*, 1997c, e). In contrast, the hydrolytic enzymes, steroid sulfatase, and β-GlcCer'ase, which are active in modifying SC lipid composition within the SC interstices, reach their maximal activity and expression coincident with formation of a competent barrier; i.e., on day 21 of gestation (Hanley *et al*, 1997c, e). Whereas hormones and other activators of nuclear receptors, which accelerate barrier maturation, induce only modest increases in the synthetic enzymes, including cholesterol sulfotransferase and glycosylceramide synthase, the corresponding, later-acting, hydrolytic enzymes, steroid sulfatase, and β-GlcCer'ase, are induced significantly by several of the hormones (glucocorticoids, T_3, estrogen) and activators (clofibrate, free fatty acids, farnesol, and JH III) (Hanley *et al*, 1997b). In contrast, androgens, which delay barrier maturation, also delay expression of the processing enzymes. Moreover, both β-GlcCer'ase and steroid sulfatase mRNA levels increase during SC maturation, paralleling the increase in enzyme activities (Hanley *et al*, 1997c, e), and implying genomic regulation of enzyme activity.

In summary, these studies demonstrate a sequence of maturational events between day 17 and day 21 in fetal rat epidermis that leads to barrier formation and SC maturation. Both Ca^{++} as well as various hormones, ligands, and activators of the nuclear receptor superfamily appear to play key regulatory roles.

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